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REVIEW OF SOIL MICROBIOLOGY PROJECTS IN THE SCI PROGRAM

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OUTLINE

I. INTRODUCTION

II. PROGRAM OBJECTIVES

III. DISCUSSION

Soil Microbiology

Program Overview

IV. RECOMMENDATIONS

V. PROJECT PROFILES

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THE PSTC-CDR SOIL MICROBIOLOGY PROGRAM

I. INTRODUCTION

This review of the A.I.D. Science Advisor's program in Soil Microbiology consisted of examining the files for those Soil Microbiology projects which have been approved for funding by the A.I.D. Program in Science and Technology Cooperation (PSTC) and the U.S.-Israel Cooperative Development Research Program (CDR). Included are projects which have been concluded and others which have just been approved for funding and little or no work has been accomplished. The files are not complete for overall projects. Some progress and final reports are not present. The comments in this review are based on the information available in the files and should not be taken as the final word as to the status or worth of the individual projects or the program as a whole.

II. PROGRAM OBJECTIVES

It is assumed that the PSTC and CDR Soil Microbiology program is intended to:

- (1) Support research using state-of-the-art techniques to advance soil microbiology theory and practice.
- (2) Support soil microbiology research that is needed for improvement of agriculture in developing countries.
- (3) Support soil microbiology research that is conducted cooperatively between developing country scientists and scientists in the U.S.*
- (4) Support soil microbiology research that will enhance the scientific capability of the developing country and is useful to the U.S.

III. DISCUSSION

Using the above assumptions the reviewer examined the project files and prepared a brief analysis of each project (attached). It was assumed that each project in the PSTC and CDR programs must stand on its own merits and that the collection of projects in soils microbiology need not add to a comprehensive program in soil microbiology, but rather it should address important problems in soil microbiology for developing countries. The following is a brief statement of the field of soil microbiology. Some areas of research that are especially in need of further research for agriculture in developing countries are identified.

* Editor's note: While the Program in Science and Technology Cooperation (PSTC) encourages U.S.-developing country scientific cooperation, this is not a necessary ingredient of projects by developed countries.

Soil Microbiology

Soil Microbiology is important in agricultural development because of the dynamic nature of the systems involved and their great influence on management practices that must be followed to obtain continued satisfactory yields of crops and maintain the natural resource. The field of soil microbiology is one of the oldest disciplines in soil science and may be thought of as consisting of four main areas of concern. They are: 1) Providing required soil nutrients, 2) Utilizing biological control mechanisms, 3) Managing soils organic matter, 4) Biodegradation of complex and simple chemical compounds.

One area of concern relating to the provision of nutrients for plants concerns N-fixation by Rhizobia. During recent years there has been a marked rise in interest in the ecology of Rhizobia and in processes associated with and factors controlling symbiotic N^2 fixation by these bacteria and legumes. Work relating to symbiotic N-fixation is especially important to developing countries who can not afford to purchase N fertilizer. Another important area of concern relates to denitrification, N-volatilization and N-leaching. Nitrogen lost through these mechanisms is not available to plants. A third general area of activity that relates to provision of plant nutrients is that of utilization of mycorrhiza to enhance availability of P and to promote plant growth.

Interest in biological control has grown from the discovery that certain soil organisms are antagonistic to other organisms and resulting inability of certain organism to grow in the soil. Fungi, bacteria, insects and weeds are the main pests of crops that require control. Examples can be cited of soil organism having adverse effects on the growth of fungi, bacteria and insects. Much remains to be done to develop biocontrol for all pests, especially weeds. The potential of biocontrol in the tropics is greater than in temperate climates in that there is a greater spectrum of plants to use if control is to be accomplished by crop manipulation.

Throughout the world, interest remains high among soil microbiologists concerning the chemistry and formation of soil organic matter and also NO^3 accumulation. Much remains to be learned in these areas. Questions concerning nitrogen mineralization and immobilization continue to be important and many issues remain unresolved. Work concerning organic matter and nitrogen transformations is a high priority in developed as well as developing countries because of the importance of NO^3 in plant growth and because of the possibility of ground water contamination by NO^3 . As interest has increased in sustainability of the soil resource for production, the importance

of maintaining soil organic matter has taken on new meaning because of the role of organic matter in soil structure and in regulating the root environment. The art of management of soil organic matter is ahead of the science. There is much that needs investigating to fully understand the dynamics of soil organic matter and to be able to maximize its beneficial roles in plant production and in maintaining the soil resource.

The field of bio-degradation in soils is taking on increasing importance as concern for the environment is growing. The speed of breakdown of both inorganic and organic compounds in the tropics can be faster than in temperate areas. The breakdown pathways, intermediate products and end products are also sometimes different in the tropics as compared to the temperate areas, therefore information cannot be readily transferred from one area to another with certainty. Frequently inorganic compounds breakdown faster than predicted and often organic compounds behave in unexpected ways as the soil environment changes. Much remains to be learned to both control and utilize bio-degradation processes in soils.

In addition to those pointed out above, there are many areas of importance in soil microbiology that are not being examined adequately. Some of these are discussed briefly below. There is a dearth of studies of the composition of the microbial community and of the ecology of major microbial groups in soils.

The role of subterranean fauna in soil transformations and chemistry has been studied very little. There is a critical need for more knowledge about the transformations of S, P and compounds of other elements that are subject to biological modifications. Other areas of study that need researching concern N^2 fixation by nodulated nonlegumes and free living or rhizosphere microorganisms, the ecology and biochemistry of the rhizosphere, interactions among microbial species, and the role of clay minerals, and microorganism's role in biochemical processes in soil formation.

It is clear from this brief description of the field of soil microbiology that much remains to be done and that much of it is of potentially great importance to developing countries.

The Agency for International Development (A.I.D.) supports work in the general field of soil microbiology through the IARCs, CRSP, S&T/AGR and the missions. It is the responsibility of these units to conduct research that is applicable to known problems confronting developing countries. On the other hand, it is the responsibility of the SCI program to conduct research using state-of-the-art technology to improve the theory and practice in soil microbiology in developing countries. It would seem that the following merit special attention in the SCI program:

- (1) Studies to improve our ability to enhance biological N-fixation.
- (2) Studies to improve our ability to increase the utilization of native soil phosphorus by plants.
- (3) Studies to improve understanding of the roles of microorganisms in creation of both desirable and toxic substances in soil.
- (4) Studies of processes which can utilize biological control mechanisms.
- (5) Studies to better understand bio-degradation processes and possibilities in the soil.
- (6) Studies to better understand the biological processes related to soil organic matter.

Program Overview

The eleven projects reviewed (see attached) address two of the four categories of soil microbiology research described above. Six of them are aimed at enhancing plant nutrient availability. Four are designed to utilize bio-control mechanisms for the benefit of plants. No projects are addressing problems concerning management of soil organic matter or bio-degradation in the soil.

Project files examined in this review included the following:

1. (2.A-04) Role of VA Mycorrhizae in Phosphorus Nutrition of Economic Leguminous Crops.
2. (2.A-32) Genetic Engineering Approach to Improvement of Rhizobia for Tropical Legumes.
3. (3.A-49) Improving Nitrogen Fixation in Grain Legumes Using Selected Strains of Rhizobia.
4. (4.088) New Techniques for Enhanced Citrus Production in Barbados and East Caribbean.
5. (5.068) Transfer of Hydrogen Uptake (Hup) Genes from Rhizobium leguminosarum to Fast-growing Salt-tolerant Rhizobium japonicum
6. (6.233) Genetic Engineering of Rhizobium to increase Efficiency of Nodulation.
7. (6.575) Mycorrhizae in the Growth and Development of Dipterocarps and Eucalyptus Seedlings.

8. (6.077) Development of Mycorrhizal Fungi as Bio-regulators of Drought Stress in Nitrogen Fixing Legumes.
9. (6.250) Microbial Antagonisms: Potential for Selecting Strains of Rhizobium to Inhibit Legume Root Pathogens.
10. (6.041) Influence of Interactions between Sulfur Cycle Reducing Bacteria and Ethanogenic Bacteria on Equilibrium of Low Lands Rice Ecosystems.
11. (4.396) Tissue Culture and Microbial Inoculation Technologies for the Improvement of Alnus Nepalensis

The projects that are addressing improvement of N Fixation by legumes includes one that is designed to select efficient strains of rhizobia for inoculation of legumes (3.A49) and three that are aimed at genetic manipulation of the rhizobium to develop a more efficient organism for inoculation, (5.068, 6.233, and 2.A32).

Efforts to identify, isolate and use the best indigenous strains of rhizobium have several advantages related to availability, competitiveness, adaptability and overall utility of locally available organism versus introduction of completely foreign organisms into the ecosystem. Progress on project 3.A49 has not been sufficient to determine if it will, in fact, provide Sri Lanka with improved rhizobia, but the odds of success seem quite high.

Theoretically, it should be possible to isolate the genes in rhizobia that are responsible for efficient nitrogen fixation and transfer them to highly competitive nonefficient strains. Projects 6.233, 2.A32 and 5.068 are designed to try to improve rhizobia through genetic engineering. They are not duplicative and they are complementary. Success in these and other similar efforts has, to date, not been very significant. Lack of success should not deter continued efforts along this line, but one should not expect early success in genetically engineering highly superior rhizobium strains. A continued modest effort in this area by SCI will complement the work of others including S&T/AGR. If any of these efforts are successful NiFTAL will develop and deliver the technology to developing countries.

The importance of BNF for developing country agriculture is so great for food, feed and fuel production and for maintenance of the natural resource base that efforts must be continued and even accelerated to develop more efficient BNF. It is recommended that the PSTC and CDR programs continue to support research in this area that meets the program criteria.

The files for six additional related projects were reviewed, but are not summarized in the attached individual project descriptions. Projects so examined are: 2.A-03, 5.114, 5.316, 5.350, 6.446, and 5.052. Each of these projects has potential to contribute to the overall knowledge bank of microbiology in soil-plant-atmosphere systems and several of them contribute to plant nutrition.

Throughout most of the world, particularly in the tropics, where highly weathered soils with highly soluble Fe, Al, and high acidity predominate, phosphorus availability for crop plants is frequently very low. Projects 6.575, 2.A-04 and 4.088 are addressing the issue of P availability. These projects utilize mycorrhizae (VAM) to modify the root system of plants and thereby increase the uptake of phosphorus. This work is highly important. The projects are complementary and not duplicative. One should expect considerable success in developing techniques and in matching VAM with plant and soil environment to enhance P uptake and plant growth. Work on mycorrhizae in developing countries should continue to be supported by SCI.

Three projects, 5.350, 6.041 and 4.396, are specifically aimed at utilizing microbial antagonism to establish and maintain a desired condition in the soil. Another project (6.077) is designed to utilize both antagonism and to modify the plant root system to enhance seedling establishment. Each of these projects has merit and can be expected to provide information that will be helpful in preventing drought stress, and in enhancing seedling establishment, water and nutrient uptake and overall crop plant growth in the environments where the work is done. It is important that this type of work be replicated in many locations so that generalizations may be drawn and prediction of results may be made as one considers various environments.

As stated above, six of the ten projects reviewed are striving to increase the supply of raw materials for plant growth--plant nutrients. They also have the potential to add valuable information to the fund of knowledge about N fixation, P availability, interactions between soil organisms and environmental effects on soil microbial processes.

It should not be expected that these projects will add a great deal of basic information about provision of plant nutrients because for the most part the results cannot easily be extrapolated to other environments. But the information gained will be applicable to the soils and the environment in which it was gathered and will be useful to the developing countries with similar situations. U.S. scientists involved will gain experience in environments not available in the U.S.

The PSTC and CDR program in soil microbiology, including the eleven projects reviewed and the six related projects examined, constitutes a group of useful projects which are addressing important issues for developing countries. The projects are generally well designed and have worthy objectives. State-of-the-art technology is being used and collaboration is being effected. These projects differ considerably in their ability to establish lasting linkages between developed and developing country scientists and to provide for strengthening the scientific capability of developing countries (see comments on each project).

It is important to note that the PSTC and CDR program addresses researchable questions concerning some organisms that are not being investigated by the S&T/AGR BNF program, thereby providing breadth to the overall A.I.D. program in soil microbiology. It is suggested that efforts to investigate questions concerning soil microorganisms other than rhizobia be continued. This does not mean that work on rhizobia should be curtailed, but rather it applauds the PSTC and CDR programs for including work on other organisms.

It should be noted that the PSTC and CDR programs do not include activities that address questions of managing soil organic matter and bio-degradation in soils. These two important components of the overall field of soil microbiology would seem to merit more attention in the future. This recommendation is made because of the importance of bio-degradation on the environment and because of the importance of soil organic matter in concerns about natural resource maintenance.

IV. RECOMMENDATIONS

Because the files of these projects are incomplete and because some projects have terminated and others are just beginning, it is difficult to evaluate these Soil Microbiology projects as a program. Therefore, each project was examined separately using the information available. Based on this limited analysis of the activities and progress of these projects in Soil Microbiology that are being supported by PSTC and CDR the following recommendations are made that:

- (1) Efforts be made to develop an overall soil chemistry program statement to guide selection of future projects in this area. Such a program statement should be designed to complement other soil chemistry activities being supported by A.I.D. and the work of others. It should emphasize state-of-the-art technology and address problems that have potential to significantly improve the food producing capacity of developing countries while maintaining the natural resource base.

(2) The PSTC and CDR programs in Soil Microbiology that are directed at providing plant nutrients be continued, but that the projects be developed so as to build networks of scientists rather than just the individual linkages that the present projects establish.

(3) The program should include projects on bio-degradation and management of organic matter. These two areas are important in the maintenance of the environment and in maintenance of the soil resource as a useable natural resource.

(4) Procedures be developed and implemented to ensure that grant holders submit interim and final project reports on time and that project holders report all Scientific Publications based on the project findings.

Project Profile

Project No.: 2.A-04

Project Title: The role of VA Mycorrhizae in the Phosphorus Nutrition of Economic Legume Crops: Soybeans, Mungbeans and Peanuts, in Thailand.

Project Duration: October 1982 - September 30, 1985

Funding Level: \$160,000

Grant Holder: Department of Agriculture, Thailand

Principal Investigator: Yenchai Vasuvat

Investigators: Dr. Omsub Nopamornbodi
Ms. Subaporn Thamsurakul
Ms. Puagpaga Arunrui

Co-Investigators:

Dr. Ben Bohlool, Univ. of Hawaii
Dr. Jake Halliday, Univ. of Hawaii
Dr. Norman Schenck, Univ. of Florida
Dr. John Menge, Univ. of California
Dr. Natakorn Boonkerd, Dept. of Agr. Thailand

Cooperating Institutions:

Department of Agriculture, Thailand
University of Florida
University of Hawaii
University of California

Project Purpose:

To increase the efficiency of production of soybeans, mungbeans and peanuts in Thailand.

Specific Objectives:

- (1) To improve the phosphorus nutrition of soybeans, mungbeans, and peanuts in Thailand.
- (2) To determine the survival of mycorrhizae following flooding of soils for rice by inoculation of studies of soybeans, mungbeans, and peanuts.
- (3) To improve the method of producing the mycorrhizal inoculum for field trials.

Discussion: (Scientific)

Spores of mycorrhizal fungi were collected from soybean, mungbean and paddy fields and divided into groups according to physical characteristics. Collected spores were multiplied in pot culture.

Ten species of VAM fungi were inoculated to soybeans and mungbeans in pot culture. The effectiveness of each species in producing spores and in colonizing roots showed that there were difference among species. Selected species were used in further experiments.

The effect of the VAM species on phosphorus absorption and growth of soybeans and mungbeans grown in pots was determined. Results indicated differences between VAM species in this respect and permitted the selection of superior species for further use in this study.

The 12 VAM species were used to inoculate soybeans and mungbeans in field plantings in three Provinces. Both soybeans and mungbeans inoculated with Glomus introardadices showed the highest yields even though there was not apparent difference in growth and plant height as compared to the check plots.

A field experiment was conducted to determine the survival of mycorrhizal fungi in flooded soils. No fungal colonization was found on the rice roots at any state of growth. The number of mycorrhizal spores in the soil was reduced by flooding.

The overall study of the effect of mycorrhizal fungi on phosphorus absorption and growth of soybeans and mungbeans indicated that in the field root colonization by mycorrhizal fungi occurred even in uninoculated plots. Mycorrhizal fungi Acaulospora scropiculata was the most effective species in the field. These results showed that percent root colonizations and number of spores in the soil will affect the yields of soybeans and mungbeans positively.

Results: (Linkages)

Leadership for this project came from the Department of Agriculture in Thailand with the cooperation of scientists from three U.S. universities. Each had specific responsibilities and were highly cooperative in the conduct of the research. Relationships established and interchanges that occurred will undoubtedly be lasting.

Results: (Improved Scientific Capacity)

Developing country:

This project strengthened the on-going research capacity of the Department of Agriculture, Thailand, not only for research on soybeans and mungbeans, but for a wide variety of crops in which mycorrhizae are important. Information gathered and techniques developed will be helpful in further studies.

U.S.:

This project strengthened by the U.S. capability to do research of this nature by giving the U.S. scientists involved experience in a different environment and by giving them access to biological material not available in this country.

Discussion:

Relation to other A.I.D. activities:

This project is complementary to A.I.D.'s objectives because it is aimed at increasing the food producing capacity of a developing country. It is also designed to strengthen the scientific capacity of a developing country. It is complimentary to S&T/AGR projects in soil fertility and microbiology and is complementary to the mission's efforts to improve the food producing and research capacity of Thailand.

Relation to other SCI activities:

When this project was initiated it was one of the first efforts by SCI to foster increased interest in mycorrhizae as a useful mechanism for enhancing phosphorus uptake by plants. Subsequent studies of this nature have been stimulated by this project.

Relation to state-of-the-art and the work of others:

When this project was initiated in 1982, it was the state-of-the-art. Subsequently, other research has refined technology for use in the studies of mycorrhizae. The literature referenced in this project was adequate and the project did not duplicate the work of others.

Project Profile

Project No.: 2.A32

Project Title: A Genetic Engineering Approach to the Improvement of Rhizobium for Tropical Legumes

Project Duration: January 1982 - December 1984

Funding Level: \$54,530

Grant Holder: University of Hawaii

Principal Investigator: B. Ben Bohlool, Microbiologist

Co-Investigator: None

Cooperating Institutions: The University of Hawaii

Project Purpose:

To increase the efficiency of indigenous strains of Rhizobia by introducing ions into the genes for efficient nitrogen fixation.

Specific Objectives:

- (1) To genetically engineer rhizobia by inserting genes from high- N-fixing, but poorly adapted strains into indigenous strains for tropical environments.
- (2) To produce "depressed" mutants of tropical rhizobia capable of fixing high levels of atmospheric nitrogen in the presence of high levels of soil nitrogen.

Research Results: (Scientific)

During the early phases of this work many cultures of rhizobium were obtained and screened for resistance to acidity and Al. High levels of Al. and pH resistance were found in a few strains. These strains were used for further studies. Transposon mutagenesis and pSym plasmid curing of CIAT 899 strain failed to produce any Al. sensitive mutants.

A series of physiological studies was conducted to try to determine the mechanism of Al. resistance in CIAT 899. This series of experiments did not provide an apparent explanation for the Al. resistance of CIAT 899. The investigations of the physiological mechanism of aluminum resistance/sensitivity did not indicate any definite mechanism operative. This avenue of research was not pursued further.

As a result of the failure of the work described above to provide useful leads, the researchers decided to take another approach. They reasoned that the pSym cured derivatives would be valuable resources to use for construction of stress resistant rhizobia for alternate host legumes. For example, CIAT 899 is an aluminum resistant bean rhizobium, by placing a pea pSym or a clover pSym into the cured derivatives it should be possible to construct rhizobia for pea and clover which are not only highly effective but are stress resistant as well.

The symbiotic properties of several transconjugants of CIAT 899' UHN-5 (M5) and several relevant backcrosses the R. leguminosarum 6015 were examined. None of these transconjugants were "Fix+" on any host plant. Transfer of plasmids pJB5JI and pBRIAN to the wild-type parent CIAT 899 resulted in transconjugants that were effective on beans, but were ineffective on the host for the introduced pSym. It was concluded that CIAT 899 has an apparent ability to rearrange the plasmid profiles. Construction of successful transconjugants requires a strain which does not rearrange to the introduced plasmids. The researchers planned to continue this work using other strains.

This work succeeded in identifying many of the difficulties in converting rhizobium strains to more effective organisms. It contributed knowledge that has been utilized by others who have attempted similar work, mostly unsuccessful to date.

Results: (Linkages)

This project did not establish any meaningful linkages with developing country scientists.

Results: (Improved Scientific Ability)

Developing Country:

There was not involvement of developing country scientists.

U.S.:

Scientists at the University of Hawaii gained valuable experience as did the graduate students who worked on this project. Although this project did not succeed in developing the improved strains that were desired, it did provide valuable information for future similar studies.

Discussion:

Relation to A.I.D. Programs:

Enhancement of nitrogen fixing ability by rhizobia will be of great benefit to developing countries. The S&T/AGR BNF program is aimed at improving BNF in developing countries. The objective of this project was compatible with the S&T/AGR effort.

Relation to other SCI programs:

This project is not duplicative of other SCI sponsored activities. It is aimed at creating new knowledge and of involving U.S. scientists. It did not involve LDC scientists or create linkages which strengthen LDC research capability.

Relation to state-of-the-art and work of others:

When this work was undertaken in 1982 it was state-of-the-art and was not duplicative of the work of others. The literature cited in the project proposal would lead one to think that the work undertaken by this project should have been successful in developing the desired organisms. Subsequent work by many other scientists has shown that the transfer of genes in rhizobium is a much more difficult task than was recognized at the time this work was initiated. This project should not be considered a failure because it showed many of the difficulties to be encountered in this type of work.

Project Profile

Project No.: 3.A49

Project Title: Improved Nitrogen Fixation in Grain Legumes Using Selected Strains of Rhizobia

Project Duration: August 1985 - July 1987

Funding Level: \$21,650

Grant Holder: University of Peradeniya, Sri Lanka

Principal Investigator: Dr. J.M.R.S. Bandara, Plant Physiology and Microbiology, Sri Lanka

Co-Investigators: Dr. D.G. Keerthisinghe
Dr. M.R. Thaigarajah

Cooperating Institutions:

Dept. of Biology, U of Peradeniya
University of Hawaii (NiFTAL)

Project Purpose:

To increase N-fixation by grain legumes in Sri Lanka through the use of selected strains of Rhizobia

Specific Objectives:

- (1) To study the effectiveness of selected indigenous strains of Rhizobia on nodulation of grain legumes in Sri Lanka.
- (2) To determine the N-fixing ability of rhizobial strains and the main biological factors affecting N-fixation.
- (3) To test the effectiveness of selected rhizobial strains on N-fixation by cowpeas and mungbeans.
- (4) To develop improved ways to produce and store Rhizobia using locally available materials.

Research Results: (Scientific)

Progress in this project was delayed by lack of glassware and chemicals. Delivery was delayed more than one year. However, the investigator did collect nodules, isolate and characterize the rhizobial strains found. Twenty (20) strains were isolated from cowpeas and nine from mungbeans. These strains were evaluated for sensitivity to a variety of environmental conditions and for vigor.

The effectiveness of the authenticated isolates of rhizobia were tested on mungbeans in the greenhouse.

To determine the effects of nitrogen status of the soil on N-fixation, field samples of soil were collected and analyzed for chemical status and physical characteristics. Both soybeans and mungbeans were grown and samples were analyzed to determine the affect of T, S, P. on N-fixation. Results were not reported.

Efforts were initiated to produce bulk culture of Rhizobia. No results were reported.

Results: (Linkages)

This project is not designed to affect cooperative work. It does, however, establish tenuous linkages with NiFTAL at the University of Hawaii.

Results: (Improved Scientific Capacity)

Developing Country:

Experience gained by scientists and students in Sri Lanka and exposure to advanced technology at NiFTAL will enhance the capacity of the Sri Lanka scientists somewhat.

U.S.:

There appears to be no benefit to U.S. science except the access to the Rhizobial strains identified in Sri Lanka.

Discussion:

Relation to other A.I.D. activities:

This project has potential to increase N-fixation of important grain legumes in Sri Lanka and thereby reduce fertilizer costs and enhance the country's ability to meet the needs for grain legumes. It is complementary to several S&T/AGR projects. Potentially, NiFTAL, SMSS, IBSNAT, and TSMM will benefit from information gained. Potential results can benefit mungbean and soybean production in other countries.

Relation to other SCI Activities:

This project has potential to complement other PSTC and CDR projects which are aimed at improving efficiency of N-fixation.

Relation to state-of-the-art and work of others:

Techniques used in the laboratory and in the field phases of this project, as planned, would be state-of-the-art. This project is similar to other work in many areas of the world where efforts are being made to identify locally available superior strains of Rhizobia. It is not innovative, but does have potentially high benefits for Sri Lanka.

Project Profile

Project No.: 4.088

Project Title: New Techniques for Enhancing Citrus Production in Barbados and the Eastern Caribbean

Project Duration: Three years

Funding Level: \$60,800

Grant Holder: USDA Agricultural Research Station, Orlando, Fl.

Principal Investigator: Stan Nemec, Plant Pathologist

Co-Investigator: Stan Michelini, Fruit Experiment Station for the Caribbean

Cooperating Institutions:

USDA/ARS Tropical Fruit Experiment Station, Orlando, Florida

Fruit Experiment Station for the Caribbean
St. James, Barbados, West Indies

Project Purpose:

To increase production of citrus in the Eastern Caribbean Islands.

Specific Objectives:

- (1) To isolate and identify vesicular-arbuscular mycorrhizae (VAM) from various citrus in plantings on the island of Barbados and two other South-eastern Caribbean Islands.
- (2) To increase and isolate native VAM species for testing the efficiency of VAM to increase nutrient uptake and growth of citrus on the islands.
- (3) Inoculate citrus in the islands with VAM from Florida that is known to enhance nutrient uptake and determine the effect on long- term longevity.
- (4) Inoculate citrus in the field with superior strains of VAM that have been isolated from the Islands and test the effects on longevity and nutrient uptake.
- (5) Supply the technical methodology and materials required to institutions and producers on other islands that request assistance.

Research Results: (Scientific)

Roots and attached soil were collected from citrus growing in the islands. Using a "trap crop" the VAM was isolated and used to inoculate sterilized media in which cleansed cuttings of citrus were planted. The results of this container experiment indicated that the VAM treated plants had significantly greater growth rate than the plants without VAM.

Detailed studies of VAM infection in native citrus groves in the islands of Dominica, St. Lucia and Grenada were made to identify the most vigorous strains of VAM.

A workshop for over 20 people was conducted in Barbados. The workshop included presentations of information of mycorrhizae, training in techniques of collection, preparation of root samples, spore collection, spore production and results of the project work to date.

A regression analysis on interactions between VAM infection and environmental factors such as soil nutrients, elevation, rainfall, and tree age showed significant correlations between several factors. Positive correlations were shown between infection and tree age, altitude, and manganese. Negative correlations were shown with the rise in pH and soil iron, sulfur, zinc, boron, and calcium.

Future plans include preparations of a scientific paper on this work, conduct of a second workshop and establishment of a 320 tree nursery for future studies will be established.

Results: (Linkages)

This project has established strong linkages between the USDA research scientists at Orlando Florida and the scientists at the Fruit Experiment Station for the Caribbean. It has also established linkages with several growers in the Islands and has trained technicians on each Island on the use of VAM to enhance the growth and longevity of citrus in the Islands.

Project Profile

Project No.: 5.068

Project Title: Transfer of Hydrogen Uptake (HUP) Genes from Rhizobium Leguminosarum to Fast-growing, Salt-tolerant Rhizobium japonicum

Project Duration: January 1, 1985 - December 31, 1987

Funding Level: \$77,227

Grant Holder: The University of Maryland

Principal Investigator: J.S. Ange, University of Maryland
S.M. Mahmoud, Assuit University, Egypt

Co-Investigator: None

Cooperating Institutions: The University of Maryland
Assuit University, Egypt

Project Purpose:

To transfer genes that confer nitrogen fixation efficiency into a salt-tolerant, inefficient strain of R. japonicum and thereby construct a R. japonicum that is capable of surviving in saline soils and fix large quantities of atmospheric nitrogen.

Specific Objectives:

- (1) Transfer hydrogen uptake (Hup) genes from Rhizobium Leguminosarum (Hup⁺) strain 3894 to USDA strain 191 R. japonicum (Hup⁻).
- (2) Determine the relative soybean nodulation potential and nitrogen fixation efficiency of R. japonicum 191 (Hup⁺) strains.
- (3) Determine whether R. japonicum 191 (Hup⁺) can increase nodulation, nitrogen fixation and soybean yield when inoculated into saline soils of Upper Egypt.

Research Results: (Scientific)

Background: Because soybeans have not been grown in Egypt there exists no indigenous soil population of R. japonicum. Therefore, soils must be inoculated before soybeans can be grown successfully. Survival of strains of R. japonicum in upper Egypt is partially limited by the high salt concentrations in the soil.

Therefore a new, salt-tolerant strain of R. japonicum is needed to permit the production of soybeans in Upper Egypt. This project proposes to transfer genes that confer nitrogen fixation efficiency into a salt-tolerant, inefficient strain of R. japonicum and test this new strain in Egypt.

A multitude of reports have established the generality of plasmid-determined host range specificity, nodulation ability, hydrogenase and nitrogenase production in fast-growing rhizobia.

This study also proposes to test the newly developed strains for competitiveness in high-salt soils of Egypt.

Experimental Procedure:

A substrain of USDA 191 R. japonicum which is fast growing and has a high level of drug resistance will be used as the host for hydrogen uptake determinants from R. japonicum strain 3894 to develop a more vigorous and efficient strain for use in Egypt. Then the new strain will be tested in Egypt.

Results: (to date)

It was found that R. fredii HH303 was more efficient than R. fredii USDA 191 on U.S. soybeans. For this reason HH303 has replaced USDA 191 on this study. An antibiotic resistant strain of HH303 has been isolated. Membrane filter matings were conducted to transfer hup plasmid pIJ1008 from R. leguminosarum 3894 to R. Ferdi HH303. The transconjugants were isolated and tested on pea plants for their relative nodulation ability and hup activity. No further results have been reported at this time.

Results: (Linkages)

The close working relationship between scientists at the University of Maryland and Scientists in Egypt will establish linkages and collegial relationships that should be lasting.

Results: (Improving Scientific Capability)

Developing Country:

Because scientists in Egypt will be working in a U.S. laboratory for an extended period of time and then will be applying the technology learned in Egypt, both in the laboratory and in the field, the capability of Host country scientists and technicians will be enhanced by this project.

U.S.:

The capability of U.S. graduate students to use the modern techniques of gene transfer and testing of the product will be enhanced by this project.

Discussion:

Relation to A.I.D. programs:

This project is aimed at developing technology that can be used to facilitate the production of important food crops in a developing country. If successful, the techniques developed would make possible production of soybeans in millions of ha. of salt affected soils in other developing countries.

This project is also compatible with A.I.D.'s aim to use and introduce new and more highly sophisticated research techniques to LDCs.

This project is also designed to facilitate the exchange of scientific information and the training of developing country scientists. It is also complementary to other A.I.D. projects such as the BNF project in S&T/AGR.

Relation to the SCI program:

This project is compatible with the SCI program aim to facilitate collaborative research and to introduce new scientific technology to developing country scientists. It is also designed to facilitate linkages and develop lasting collaboration between U.S. scientists and their counterparts in developing countries.

Relation to state-of-the-art and work of others:

This project proposal includes an unusually complete summary of relevant literature. It is clear that the technology proposed for use in this project is the state-of-the-art and that the work to be undertaken is addressing an important problem in a developing country and is not duplicative of work being undertaken elsewhere.

Project Profile

Project No.: 6.233

Project Title: Genetic Engineering of Rhizobia to Increase Nodulation Efficiency

Project Duration: January 1986 - December 1988

Funding Level: \$150,000

Grant Holder: Battelle-Kettering Laboratory

Principal Investigator: T. V. Buvaneswari, Research Scientist
Battelle Laboratory

Co-Investigators:

S.J. Vesper, Battelle Lab.

M.H. Ahmad, Biochemistry
University of the West Indies, Jamaica

Cooperating Institutions: Battelle-Kettering Laboratories, Ohio
University of the West Indies, Jamaica

Project Purpose:

To determine the genetic basis of the nodulation efficiency in Rhizobium japonicum strain 62A76, isolate the genes involved, and introduce them into other strains of R. Japonicum.

Specific Objectives:

- (1) To construct a gene bank of R. japonicum strain 61A76 into a broad host range vector, pRK293 and to isolate the genes that determine the efficiency of nodule formation (Nef genes) in soybean and cowpea.
- (2) To introduce the isolated efficiency genes into other strains of R. japonicum for use in Jamaica as inoculants.

Research Results: (Scientific)

The research plan, in brief, is as follows:

To isolate the genes that determine the nodulation efficiency (Nef genes) a gene bank of the efficient slimy phenotype will be developed. The cloned fragments will be introduced into the inefficient variant and the efficient transconjugants will be

selected. Efforts will be made to introduce the isolated Nef genes into other R. japonicum strains in Jamaica and compare the nodulation efficiency of transconjugants with that of the parent.

Isolates obtained from Jamaican soil that show superior nitrogen fixing capabilities on local varieties of soybeans and cowpea will be used by researchers in Jamaica. R. japonicum strains commonly used in the U.S. will be used for experiments in the U.S.

Results: (to date)

One of the objectives of this project is to understand the genetic basis of the slimey variant in the mixed rhizobium colony. Preliminary results indicate that strain 61A76 possesses high intrinsic resistance to tetracycline, a marker in the original vector. The original vector was reconstructed to contain the NPT gene.

A variant 61A76 that possesses only the slimey compact phenotype has been obtained, but failed to nodulate soybeans. They also failed to deform root hairs. Experiments are underway to mate the gene bank at Kettering into these variants and select the recombinants from the mixed colony according to Nod phenotype. These experiments will identify the clones that possess the genes present in the parent that are responsible for its mixed colony phenotype.

No field results have been reported.

Results: (Linkage)

Since this research is conducted both at Battelle and at the University of West Indies involving an existing group of scientists and students at each institution who are working on problems of nitrogen fixation, this work should facilitate collaboration. Persons from Jamaica will be involved in planning, initiation, and conducting every phase of this study.

Results: (Improvement of Scientific Capability)

Developing Country

Involvement of scientists and students from a developing country university with a cutting-edge research organization in the U.S. will undoubtedly increase the scientific capability of the persons involved.

U.S.:

Exposure of U.S. scientists to problems in a developing country will broaden their horizons and provide them with materials for research that are not available in the U.S.

Discussion:

Relation to other A.I.D. Programs:

This activity addresses a serious problem in many developing countries where nodulation of legumes is less than satisfactory. Increasing the efficiency of nodulation and nitrogen fixation of nitrogen by legumes would greatly benefit many developing countries by reducing the cost of imported nitrogen fertilizer and by increasing yields of important food crops. This project is complementary to other SCI projects aimed at the same problem and to the S&T/AGR program on BNF.

Relation to other SCI projects:

This project is soundly conceived and is highly complementary to other SCI projects that address the problem of nitrogen fixation by legumes in developing countries.

Relation to state-of-the-art and work of others:

The literature review accompanying the project proposal shows clearly that this activity is state-of-the-art and is not duplicative of the work of others. It will contribute important information on techniques for isolation of genes and incorporation of these genes in other strains of bacteria. It will also test the hypothesis that competitiveness and efficiency of R. japonicum strains can be enhanced in acid tropical soils.

Project Profile

Project No.: 6.575

Project Title: Development of Direct Seedling and Bare Rooting Techniques for Eucalyptus and Dipterocarps Using Ectomycorrhizal Fungi

Project Duration: September 1985 -August 1989

Funding Level: \$17,772

Grant Holder: University of the Philippines, Los Banos

Principal Investigator: Reynaldo E. de la Cruz, Department of Forest Biology UPLB

Consultants: Donald H. Marks, USDA Forest Service, Athens, GA
J. M. Trappe, Corvallis, Oregon

Cooperating Institutions:

University of the Philippines, Los Banos
Oregon State University
USDA Forestry Service

Project Purpose:

To develop a means to increase the survival and growth of Eucalyptus and Dipterocarps.

Specific Objectives:

1. To determine the feasibility of using ectomycorrhizal fungi in promoting growth of seedlings.
2. To identify the ectomycorrhizal fungi associated with Eucalyptus and Dipterocarps.
3. Test different inoculation techniques for massive production of mycorrhizal seedlings of Dipterocarps and Eucalyptus.
4. To test the feasibility of using mycorrhizal fungi in developing a bare-root planting technology for Eucalyptus and Dipterocarps seedlings and direct-seeding technology of Dipterocarps seedlings.

Research Results: (Scientific)

None reported to date.

Project Plan: (In brief)

Projected project activities include training for the principal investigator in the U.S. on mycorrhizal identification and identification and isolation of mycorrhizal material from established tree nurseries and forests in the Philippines. This work will be followed by use of mycorrhizal isolates to treat seedlings of Eucalyptus and Dipterocarps which will then be planted in experimental pots and field plots for study of the effects of treatment on survival and growth.

Results: (Linkages)

Collaboration between the principal research and consultants in the U.S. will establish linkages that should be continuing.

Improved Capability:

Developing Country:

Involvement in sophisticated research using Mycorrhiza will increase the knowledge and capability of the persons involved in the Philippines.

U.S.:

Being associated with this work in the Philippines and having access to the data produced will add to the knowledge base of the U.S. scientists involved.

Discussion:

Relation to A.I.D. programs:

This project is highly relevant to A.I.D.'s efforts to facilitate reforestation efforts in developing countries. Several developing countries are investing heavily in reforestation efforts. Methods to increase seedling survival and increase growth will pay handsome dividends for developing countries.

Results from this project will be complementary to efforts of S&T/FENR and to the efforts of the Agency to affect agroforestry in developing countries.

Relation to SCI programs:

This project is not duplicative of other efforts supported by SCI. It is complementary to those efforts that are aimed at efficient and effective use of fertilizers, especially P fertilizers.

Relation to state-of-the-art and the work of others:

The complementarity of mycorrhizal fungi and plant is well known. The fungi have been used in numerous studies which have shown that uptake of P is enhanced and a more vigorous plant result. Mycorrhiza has been used in pot and greenhouse work extensively. The innovative character of this project rests in its attempt to develop a technology for incorporating mycorrhiza in bare-root planting of Eucalyptus and Dipterocarps and in direct seeding of Dipterocarps. Success will greatly facilitate reforestation work and save great amounts of cost.

Project Profile

Project No.: 6.077

Project Title: Mycorrhizal Fungi as Bio-regulators of Drought Stress in Nitrogen Fixation

Project Duration: 3 years (awaiting funding)

Funding Level: \$149,312

Grant Holder: USDA/ARS

Principal Investigator: Gabor J. Bethlenfalvay, USDA

Co-Investigators: Robert N. Ames, USDA
K. R. Krishna, Microbiologist, ICRISAT

Cooperating Institutions: USDA/ARS
ICRISAT

Project Purpose:

To develop and utilize VAM fungi as a new technology in regulating drought stress in nodulating legumes, to achieve greater crop productivity and water-use efficiency.

Specific Objectives:

- (1) To determine the factors which influence vesicular arbuscular mycorrhizal (VAM) fungi to improve legume water stress resistance.
- (2) To determine the effects of increased legume drought tolerance due to colonization by VAM fungi on N-fixation, photosynthesis, phosphorus nutrition, water status and productivity of legumes.

Research Results: (Scientific)

Results have not been reported to date.

Research Plan: (in brief)

The project purposes and objectives will be pursued by examining the following hypothesis:

- (1) VAM fungi or VAM infected root systems can take up water not available to non-VAM infected root systems.

- (2) There are genetic differences in the ability of VAM fungi to take up bound water in the soil.
- (3) Successful introduction of a new crop species into a stressful environment depends, among other things, on the presence or absence of co-introduction of compatible VAM fungi.

Results of other studies of fungi-plant symbiosis led to the development of the above hypothesis. To test the hypothesis, both VAM (several strain) and non-VAM plants will be grown unstressed and in drought stress conditions. Factors such as N fixation, photosynthesis, and root respirations will be measured. Field experiments on several soils at ICRISAT will be conducted.

Results: (Linkage)

This joint research with scientists at ICRISAT will give both U.S. and ICRISAT scientists experience in joint efforts between the two organizations. It will not establish direct linkages with developing country scientists.

Improved Scientific Capacity:

Developing Country:

Scientific capacity of a developing country will be enhanced only as the ICRISAT scientists involved interact with colleagues in LDCs.

U.S.:

Experience gained by U.S. scientists in working with an overseas research organization will enrich the U.S. scientific capability.

Discussion:

Relation to other A.I.D. programs:

This project will be significant to the extent that it contributes to developing country ability to cope with drought and to enhance N-fixation. Both coping with drought and N-fixation are important A.I.D. objectives.

This project complements S&T/AGR's BNF, and soil and water management efforts. Many mission programs include projects that will benefit from the research if it is successful. An example is the Jordan Highlands Project.

Relation to other SCI projects:

This project complements other projects in the PSTC and CDR portfolio that address the use of mycorrhizal fungi and N-fixation. Information gained will likely complement results of other projects also.

Relation to state-of-the-art and work of others:

It is known that VAM fungi affect P and water availability. It is also known that P and water availability are interdependent as drought stress aggravates P stress.

The answers to questions addressed in this project will constitute new knowledge of great potential importance to many developing countries. It utilizes state-of-the-art technology and does not duplicate the work of others.

Project Profile

Project No.: 6.250

Project Title: Microbial Antagonism: The Potential for Selected Strains of Rhizobium to Inhibit Legume Root Pathogens

Project Duration: January 1986 - December 1989

Funding Level: \$57,600

Grant Holder: The University of Virginia

Principal Investigator: Linda Lennox Blum, Univ. of Va.

Co-Investigator: Carlos Ramirez, Universidad de Costa Rica

Cooperating Institutions:

Department of Environmental Sciences
University of Virginia

Facultad de Agronomia
Centro de Investigaciones Agronomicas
Universidad de Costa Rica

Project Purpose:

To investigate the potential of selected Rhizobium strains to protect legume seedlings from Rhizoctonia damping-off.

Specific Objectives:

- (1) To select Rhizobium strains that are capable of decreasing the incidence of Rhizoctonia damping-off and test these strains under laboratory conditions.
- (2) To field test Rhizobium strains that show the greatest potential for decreasing incidence of disease.

Research Results: (Scientific)

A large number of strains from the U.S. and Costa Rica of both R. phaseoli and R. solani have been isolated. Eighty (80) R. phaseoli and 18 R. solani strains have been randomly selected for use in screening tests of antagonistic activity.

Several techniques for evaluating the antagonistic effect of bacteria on fungi show that the results tend to be consistent for

some strains but not for others. Further work is needed to establish the best evaluation method or methods. The work plan of this project is proceeding according to schedule.

Results: (Linkages)

The close working relationship established and the jointness of effort required by this project will undoubtedly forge lasting relationships between the individuals involved and the institutions they represent.

Results: (Improved Scientific Capacity)

Developing country:

Experience gained by personnel in Costa Rica in sophisticated laboratory and field experimental work will increase capability for future work.

U.S.:

Experience gained by scientists and students in the U.S. while working on this collaborative project will increase their ability to conduct similar work in the future.

Discussion:

Relation to A.I.D. programs:

This project is in harmony with A.I.D.'s aim to help developing countries increase their food producing capabilities. Rhizoctonia damping-off which kills seedlings results in decreased food production in many developing countries. Control of this disease with chemicals is expensive and antagonistic to nitrogen fixing organisms. The aim of this project to biologically control Rhizoctonia and simultaneously enhance nitrogen fixation is a worthy goal.

This project is in harmony with S&T/AGR's BNF activities. It is also in harmony with A.I.D.'s efforts to reduce the amount of pesticides applied in developing countries.

Relation to SCI program:

This project is a good example of a project that affects true collaboration between U.S. and LDC scientists and students. It is also in harmony with the SCI aim to direct funds to important scientific questions that have potential to help developing countries. This project is not duplicative of other activities sponsored by SCI.

Relation to state-of-the-art and work of others:

The use of biological agents to control plant diseases is not new or innovative. However, the use of microorganism that enhance plant growth to control a plant disease is a new approach to biological control. It is not duplicative of other known work.

Project Profile

Project No.: 6.041

Project Title: Influence of the Interaction Between Sulfur Cycle
Reducing Bacteria and Methanogenic Bacteria on the
Low Lands Rice Production

Project Duration: July 1985 - June 30, 1988

Funding Level: \$125,500

Grant Holder: Institut Superieur Polytechnique (ISP), University
of Ouagadougou, Burkina Faso

Principal Investigator: Alfred S. Traore, Prof. of Biochemistry
and Microbiology

Co-Investigators:

Mpuhpussome Nacro, Professor of Chemistry

Bougini Pare Professor of Soil Science

Babine A. Kanwe, Professor of Biochemistry

Collaborators:

J. LeGall, Professor of Biochemistry, University of Georgia

Harry D. Peck, Jr., Prof. Dept of Biochemistry, Univ. of Georgia

William B. Whitman, Asst. Professor of Microbiology, University
of Georgia, Jose J. J. G.

Moura, Associate Prof. of Biochem. Universidad Nova De Lisboa,
Lisbon.

Cooperating Institutions:

The University of Ouagadougou, Burkina Faso

The Universidad Nova De Lisboa, Portugal

The University of Georgia, U.S.A.

Project Purpose:

To improve low land rice productivity by microbial control of
sulfide and acetate accumulation.

Specific Objectives:

- (1) Evaluate sulfide toxicity in lowland rice fields using microbial tests based on the interactions between sulfur cycle reducing bacteria and methane producing bacteria.
- (2) Investigate the physiology and biochemistry of the interaction of these two types of bacteria in lowlands ecosystem.
- (3) Investigate the use of methanogens and/or sulfate reduction inhibitors to reduce sulfate production in rice fields and improve nitrogen fixation.

Research Results: (Scientific)

No results reported to date.

The research plan, in brief, is as follows:

The aim of preventing soil toxification by microbial sulfide and the restoration of productivity of toxified soils and improvement of nitrogen fixation in lowland soils used for rice production is being pursued by the introduction of methanogens to compete with the sulfur reducing organisms. The plan includes both laboratory and field studies using selected species of organisms under both controlled and field conditions. The project appears to be well designed and manned by capable people.

Results: (Linkages)

This project is designed to affect close collaboration between scientists and the University of Georgia, the Universidade Nova De Lisboa and the ISP in Burkina Faso. It should result in continuing collaboration long after this project is completed.

Results: (Improvement of Scientific Capability)

Developing Country:

The project plan provides for equipping a laboratory in Ouagadougou. It will also permit capable scientists in Ouagadougou to continue to work on fundamental problems utilizing modern methods to attack problems of importance in their own country.

U.S.:

Exposure of U.S. Scientists to important microbial problems in a developing country will enhance their level of scientific competence.

Discussion:

Relation to other A.I.D. programs:

This project is compatible with A.I.D. objectives of increasing food production capacity and removing productivity obstacles in developing countries. It is complementary to the work at IRRI and WARDA which A.I.D. supports. It is also complementary to soil fertility and soil management efforts in S&T/AGR.

Relation to other SCI projects:

This project is a logical addition to the soil microbiology program of SCI. Sulfur chemistry in soils is important in many wetland areas of the world.

Relation to state-of-the-art and the work of others:

The basis for this study is the possible competition between sulfate-reducing organisms and methanogens for the substrate acetate. The chemical reactions involved are common knowledge, but the dynamics of the two reactions in the soil have not been extensively investigated. This study is innovative in that it will bring a new and handy way of controlling sulfur reduction in rice soils and permit the restoration of rice fields that have been abandoned. Scientifically this project will contribute to more information about methanogenesis in rice fields. It does not appear to be duplicative of the work of others.

Project Profile

Project No.: 4.396

Project Title: Tissue Culture and Microbial Inoculation
Technologies for the Improvement of Alnus
Nepalensis

Project Duration: April 1985 - March 1987

Funding Level: \$125,000

Grant Holder: Native Plants, Inc., Salt Lake Utah

Principal Investigator: Stephen Garton

Co-Investigator: None identified in the proposal

Cooperating Institutions:

Native Plants, Inc.

Ministry of Forest and Soil, Nepal

Ministry of Food, Agriculture and Irrigation, Nepal

Project Purpose:

To increase survival of planting stock and enhance reforestation and thus increase wood supplies in Nepal.

Specific Objectives:

- (1) To improve the availability of superior stock of Alnus nepalensis.
- (2) To improve the stand establishment and growth rate in A. nepalensis by inoculating planting stock with superior strains of actinorhizal symbiont, (Frankia).
- (3) To enable scientists in Nepal to apply such techniques as microbial inoculation and plant tissue culture to large scale tree production programs.
- (4) To gain a more complete understanding of alder-Frankia symbiosis.

Research Results: (Scientific)

The project proposal is well designed and presents a logical and worthwhile plan of action. To date no results are reported.
The project work plan includes the following:

- (1) A collection of plant material (seed) will be carried out in the U.S. and Nepal. Vegetative cuttings will be made from superior trees in Nepal. In Vitro cultures will be used to establish stock plants in both the U.S. and Nepal.
- (2) In Vitro culture will be used to broadly screen for superior materials.
- (3) An in vitro multiplication system which will provide stock cultures for further multiplication and a source of planing stock will be developed.
- (4) Shoots of microcuttings developed in the steps above will be cultured for survivability under several environments.
- (5) Tissue cultured plants will be inoculated with superior Frankia strains and plants will be grown to determine the best transplant media to ensure rapid colonization of the A. nepalensis root systems.

Results: (Linkages)

During the course of this research, Nepali cooperators will visit NPI and NPI scientist will visit Nepal. Training will be given Nepali scientists in plant tissue culture methods and in identification and collection of plant material. During these exchanges bonds will be developed between scientists that should extend beyond the life of this project.

Results: (Improved Scientific Capacity)

Nepali scientists will gain experience and develop skills not previously acquired. This training and experience will enhance Nepal's capability to conduct similar research with other plants.

U.S.:

Experience gained by U.S. scientists in Nepal will increase their sphere of knowledge and facilitate future research in this and other areas.

Discussion:

Relation to other A.I.D. activities:

This project address a critical need in Nepal and contributes to A.I.D.'s objective to reforest fragile land, develop wood supplies, and protect the natural resources. It especially contributes to the S&T/FENR program and will add to S&T's knowledge of tissue culture and use of organisms to enhance plant

establishment and liveability. It also compliments S&T/AGR activities related to tissue culture of other plants.

Relation to other SCI activities:

This project complements other PSTC and CDR projects that have objectives aimed at better understanding of the interactions in the soil-plant-environment complex. It also complements other tissue culture activities and is the only one dealing with woody species in the PSTC-CDR portfolio.

Relation to state-of-the-art and the work of others:

This project uses known technology. It is innovative in that this technology will be used by developing country scientists on woody species of considerable importance in Nepal. It applies state-of-the-art technology in a developing country and is complementary to on-going work in the U.S. and several other developed countries.

DISTRIBUTION:

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Project files, project officers and principal investigators of:

2.A-04

2.A-32

3.A-49

4.088

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2.A-03

5.052

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